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Short Communication

Boosting the electrocatalytic activity of *Desulfovibrio paquesii* biocathodes with magnetite nanoparticles



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ABSTRACT

The production of reduced value-added chemicals and fuels using microorganisms as cheap cathodic electrocatalysts is recently attracting considerable attention. A robust and sustainable production is, however, still greatly hampered by a poor understanding of electron transfer mechanisms to microorganisms and the lack of strategies to improve and manipulate thereof. Here, we investigated the use of electrically-conductive magnetite (Fe_3O_4) nanoparticles to improve the electrocatalytic activity of a H_2 -producing *Desulfovibrio paquesii* biocathode. Microbial biocathodes supplemented with a suspension of nanoparticles displayed increased H_2 production rates and enhanced stability compared to unamended ones. Cyclic voltammetry confirmed that Faradaic currents involved in microbially-catalyzed H_2 evolution were enhanced by the addition of the nanoparticles. Possibly, nanoparticles improve the extracellular electron path to the microorganisms by creating composite networks comprising of mineral particles and microbial cells.

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Introduction

Microbial electrosynthesis, that is the conversion of electricity into reduced value-added chemicals or fuels using microorganisms as catalytic agents, is attracting considerable attention as a method to store electrical energy generated from

renewable, yet intermittent, sources (e.g. sunlight and wind) [1–3].

Key to microbial electrosynthesis is the capacity of certain microorganisms to reduce inorganic compounds (e.g. carbon dioxide or protons) into stable, energy dense molecules (e.g. methane, acetic acid, formate, hydrogen) with solid-state electrodes (cathodes) serving as direct or indirect electron

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donors in their energy metabolism [4,5]. Compared to conventional chemical cathodes, microbial biocathodes have, at least in principle, the advantage of being less expensive and self-regenerating [5].

A robust and sustainable production of reduced value-added compounds with microbial biocathodes is, however, still greatly hampered by a poor fundamental understanding of the involved extracellular electron transfer (EET) mechanisms, from electrodes to microorganisms, and the lack of strategies to improve and manipulate thereof [6,7].

Recent studies have shown that, in mixed cultures, electrically-conductive magnetite nanoparticles accelerate syntrophic or cooperative metabolisms by promoting interspecies (microbe-to-microbe) EET processes [8–11]. In such studies, it was proposed that nanoparticles serve as electron conduits between electron-donating and electron-accepting microorganisms. Other studies have shown that addition of magnetite nanoparticles to an acetate-oxidizing microbial bioanode enhances anodic current generation by facilitating EET from microorganisms to the electrode [12]. So far, the application of nanoparticles to improve the performance of microbial biocathodes for hydrogen production has not been addressed yet. Here, we have explored the addition of magnetite nanoparticles as a strategy to enhance and manipulate EET in a H₂-producing microbial biocathode based on the model organism *Desulfovibrio paquesii* which has been previously shown to catalyze H₂ generation via direct EET [13].

Materials and methods

Strain, medium, and culture conditions

D. paquesii was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Culture media preparation, growing conditions and inoculation procedure were thoroughly described in a previous publication [13]. In brief, *D. paquesii* was pre-grown at 25 °C in 250 mL (total volume) sealed serum bottles containing 150 mL of anaerobic medium supplemented with lactate (10 mM) and sulfate (5 mM), under a CO₂/N₂ (30/70% v/v) atmosphere. Stationary-phase (10-day old) cultures were employed as inoculum for the bioelectrochemical experiments, hereinafter described. To this aim, an aliquot (50 mL) of the culture was anaerobically transferred to the cathode compartment of the bioelectrochemical cell.

Synthesis of magnetite nanoparticles

Magnetite nanoparticles were synthesized by chemical coprecipitation of Fe²⁺ and Fe³⁺ ions in an alkaline (1.5 M NaOH) aqueous solution, according to a procedure described previously [11,14]. Prior to being spiked to the bioelectrochemical cell, the aqueous suspension of nanoparticles was filtered across a hydrophilic membrane (pore size 0.2 μm), in order to eliminate particles and aggregates having a diameter larger than 200 nm. Total Fe concentration in the filtered suspension was approximately 80 mg/L.

Bioelectrochemical cell

All bioelectrochemical experiments were carried out in a two-compartment bioelectrochemical cell, consisting of two gastight borosilicate bottles separated by a 3 cm² Nafion® 117 proton exchange membrane. The working electrode (cathode) was a graphite rod (6 mm diameter, Sigma Aldrich, Milano, Italy), with a nominal surface area of 9.7 cm² (calculated by taking into account only the part of the electrode that was immersed in the liquid phase). The counter electrode (anode) was a glassy carbon rod (5 mm diameter, HTW GmbH, Germany). A KCl saturated Ag/AgCl reference electrode (+199 mV vs. standard hydrogen electrode, SHE) (Amel S.r.l., Milano, Italy) was also placed in the cathode chamber, assisted by a Luggin capillary. The electrolyte solution, both at the cathode and at the anode, consisted of anaerobic medium, unless otherwise indicated. While the target cathode reaction was H₂ evolution, the anode reaction was water oxidation. Additional information regarding the experimental setup was included in previous publication [13]. Throughout the manuscript all potentials are reported vs. SHE.

Bioelectrochemical experiments

All the electrochemical measurements and experiments were carried out using a VSP potentiostat (Bio-logic, Claix, France), as described elsewhere [13]. For chronoamperometric tests, the working electrode (i.e. cathode) was polarized at –900 mV vs. SHE and the current was recorded over time. Both abiotic (control) and biotic chronoamperometric tests were conducted. For the biotic tests, the cathode compartment contained either 80 mL of anaerobic medium, 50 mL of *D. paquesii* culture and 20 mL of filtered (0.20 μm) suspension of magnetite nanoparticles (*D. paquesii* + magnetite test) or 80 mL of anaerobic medium, 50 mL of *D. paquesii* culture and 20 mL of filtered (0.20 μm) de-ionized water (*D. paquesii* test). Biotic tests (containing or not magnetite nanoparticles) were always set up in parallel using the same inoculum in order to eliminate bias due to the possible presence residual lactate traces in the inoculum, as well as differences among inocula in terms of cell density and activity. For the abiotic tests, the cathode compartment contained 130 mL of anaerobic medium plus 20 mL of filtered suspension of magnetite nanoparticles (magnetite test) or simply 150 mL of anaerobic medium. Regardless the type of test being conducted, at regular intervals (e.g. every 2 h), gaseous samples were removed from the headspace of the cell using a gastight syringe (Hamilton, Reno, USA) and analyzed by gas-chromatography for hydrogen, as described previously. H₂ production calculations were performed measuring the increase in concentration in the headspace of the cell. During tests, the cell was maintained at 25 °C in a water bath, under vigorous magnetic stirring to ensure that current generation was not substantially affected by mass transfer phenomena. The cumulative electric charge (expressed as microequivalents, μeq-*i*) that was transferred during the test was calculated by integrating the current (A) over the period of electrode polarization. Cumulative equivalents (μeq-H₂) that were used for the formation of H₂ were calculated from the measured amounts of H₂, considering the corresponding molar conversion factor of

2 $\mu\text{eq}/\mu\text{mol}$. Coulombic efficiency (CE) for H_2 was accordingly calculated as $\text{CE} (\%) = (\mu\text{eq-}\text{H}_2/\mu\text{eq-}i) \times 100$. For cyclic voltammetry experiments, the electrode potential was varied in the range +200 mV to -1000 mV vs. SHE; cyclic voltammograms were recorded at different scan rates, from 1 mV/s to 100 mV/s.

Analytical methods

H_2 was analyzed in 500 μL gaseous samples by a Varian 3400 gas-chromatograph (stainless-steel column packed with molecular sieve, Supelco, He carrier gas 18 mL/min; oven temperature 180 $^\circ\text{C}$; thermal-conductivity detector (TCD) temperature 200 $^\circ\text{C}$).

Results and discussion

The effect of magnetite nanoparticles on the electrocatalytic activity of the *D. paquesii* biocathode was initially assessed by cyclic voltammetry (CV) (Fig. 1).

Fig. 1(a) compares typical cyclic voltammograms (scan rate: 10 mV/s) of the *D. paquesii* biocathodes in the presence (green line) and in the absence (red line) of magnetite nanoparticles. For comparative purposes the CV profile of a magnetite-supplemented graphite electrode in the absence of microorganisms, is also shown (blue line). The tests were carried out after the bioelectrochemical cells had been continuously polarized at -900 mV, for a period of 6 days. In the presence of *D. paquesii*, the cathodic current started to increase for potentials lower than -750 mV, reaching at -1000 mV a value of -0.46 mA/cm², that is over 3.5-times higher than that observed with graphite electrodes that were not inoculated (both in the presence and in the absence of magnetite nanoparticles). This finding confirms the previously reported ability of *D. paquesii* to catalyze H_2 generation with a graphite electrode serving a direct electron donor for H^+ reduction [13,15–17].

In the biocathode that was supplemented with magnetite nanoparticles, the onset potential for cathodic current generation remained apparently unchanged (i.e. -750 mV); however, a much steeper increase of the current was observed with a value of -1.06 mA/cm² being reached at -1000 mV.

While no other major redox processes were observed during the cathodic sweep of the tested biocathodes, a number of positive current peaks were observed with the inoculated electrodes during the following anodic sweep, particularly in the range from -650 mV to $+300$ mV. The nature and role of these peaks could not be determined in this study. However, considering that hydrogenases are known to catalyze reversibly both hydrogen evolution (from proton reduction) and hydrogen oxidation, it is possible that some of these peaks corresponded to the (bio)oxidation of H_2 . On the other hand, the midpoint potential of these peaks is also compatible with that of c-type cytochromes, which are known to be abundant in *Desulfovibrio* species [7]. Remarkably, the peaks which appeared during the anodic sweep also displayed a slightly greater intensity in the presence of magnetite nanoparticles, thereby suggesting that, regardless the nature of the enzyme giving rise to the electrochemical signal, the underlying redox reaction was accelerated by the presence of conductive nanoparticles.

In order to gain a deeper understanding of the electron transfer sites possibly involved in H_2 evolution by *D. paquesii*, the first derivative of the cathodic current was computed (Fig. 1(b)). Two peaks, one centered at -810 mV (E_{f1}) and one centered at -890 mV (E_{f2}), appeared in the *D. paquesii* biocathode, whereas no appreciable peaks were detected (in the window of cathode potentials relevant to H_2 evolution) in the non-inoculated control in the presence of magnetite nanoparticles, thereby providing a strong indication of the biological nature of the observed redox signals. Remarkably, the *D. paquesii* biocathode supplemented with magnetite nanoparticles also displayed two peaks, partially unresolved, having nearly the same formal potentials, but a much greater intensity (particularly that centered at -860 mV) than those observed in the non-amended *D. paquesii* biocathode. Cyclic voltammograms recorded at different scan rates (from 1 mV/s to 100 mV/s) revealed that cathodic peak current displayed an almost linear relationship with the scan rate ($R^2 = 0.98$), thereby suggesting that the redox process was confined to the electrode surface.

Taken as whole, the results of CV indicate that electrically-conductive magnetite nanoparticles do not exhibit an intrinsic electrocatalytic activity towards H_2 evolution; rather they magnify the intensity of

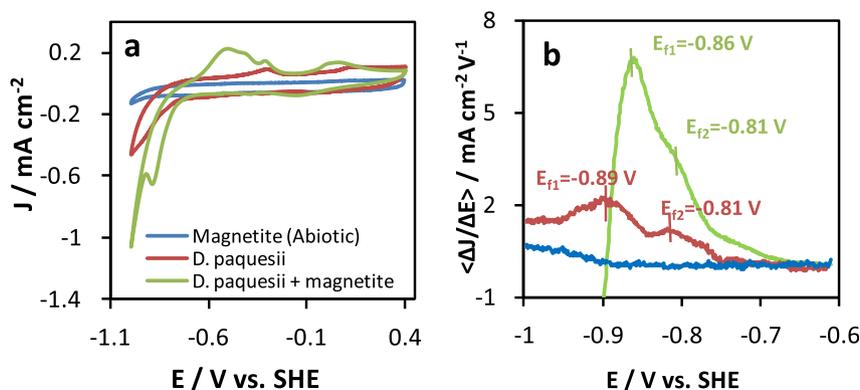


Fig. 1 – (a) Cyclic voltammetry (scan rate: 10 mV/s) of the different (bio)cathodes. (b) First derivative of the cathodic current. The formal potential of main redox peaks is also indicated.

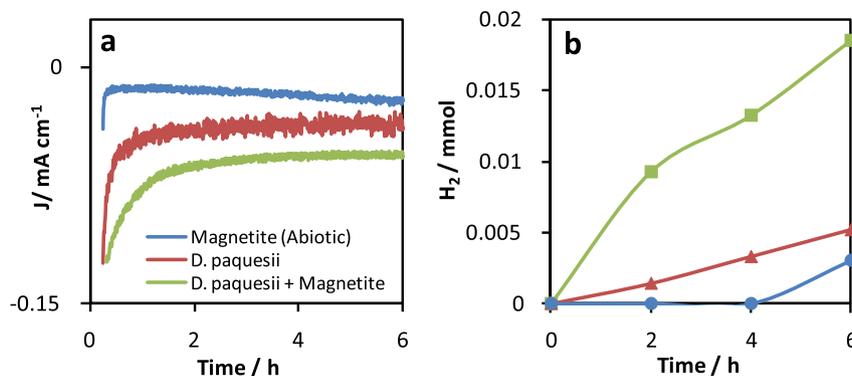


Fig. 2 – Results of 6-h chronoamperometry batch tests carried out on the different (bio)cathodes at -900 mV. (a) Current and (b) hydrogen generation over time.

bioelectrocatalytic processes induced by the presence of *D. paquesii* at the electrode.

In order to verify whether the magnetite-driven enhancement of cathodic current (observed in CV tests) ultimately corresponded to an increased H_2 generation, chronoamperometric (CA) batch tests were performed (Fig. 2). For these tests, the working electrode was potentiostatically controlled at -900 mV, a value slightly more reducing than the formal potential of the redox process that appeared to be mostly affected by the presence of magnetite nanoparticles.

Steady-state current outputs in CA tests followed the same trend already observed in the CV tests. Specifically, current density in the *D. paquesii* biocathode supplemented with magnetite nanoparticles (approx. -0.055 mA/cm², after 6 h of polarization, Fig. 2(a)) was substantially higher than in the non-amended *D. paquesii* biocathode (approx. -0.035 mA/cm²), and in the abiotic control supplemented with magnetite nanoparticles (approx. -0.022 mA/cm²). Importantly, current generation in CA tests was linked to H_2 evolution (Fig. 2(b)), with current efficiencies (i.e. conversion yields of electric current into H_2) typically in the range 15–35%.

To explore the impact of magnetite nanoparticles on the resilience of the *D. paquesii* biocathodes, CA tests were carried out on the different cells, after 1 and 6 days of continuous polarization at -900 mV (Fig. 3).

The cumulative electric charge transferred over the 6-h CA batch tests was used as a parameter to compare the activity of the different cells over time. Over the 6-day operational period, the electrocatalytic activity of the biocathode supplemented with magnetite nanoparticles decreased by approximately 16%. Indeed, a charge of 5.2 mA h was transferred during the test carried out on day 1, whereas a charge of 4.4 mA h was transferred on day 6. By contrast, the activity of the non-amended biocathode decreased by over 45%, thereby confirming that the addition of magnetite nanoparticles provided a greater electrocatalytic stability and resilience to the *D. paquesii* biocathode. Clearly, longer-term investigations are needed to confirm the improved durability and sustainability of the magnetite-supplemented biocathode. Furthermore, strategies to immobilize magnetite particles at the electrode surface should also be devised. Along this line, a recent investigation by Confocal Laser Scanning Microscopy (CLSM)

has revealed the occurrence of close associations between microbial cells and magnetite nanoparticles, with these latter being incorporated within microbial aggregates [18]. This finding raises the possibility that complex and expensive immobilization methods are not necessary to retain the particles at the electrode surface.

With uninterrupted polarization, on day 8, the non-amended biocathode that had lost most of its catalytic activity was supplemented with magnetite nanoparticles and a new CA batch test was then carried out. Interestingly, upon addition of magnetite nanoparticles, the electrocatalytic activity of the biocathode (in terms of charge production) was fully recovered (Fig. 3). This finding provided a clear indication that the time-dependent reduction of activity was not due to an irreversible decrease of microbial activity (which could not be otherwise recovered) but rather to the likely occurrence of passivation phenomena (e.g. absorption of non-conductive material over the electrode) hampering the capacity of bacteria to engage in EET with the electrode and other cells. Most probably, freshly added magnetite nanoparticles allowed creating a composite network made of bacterial cells and electrically-conductive mineral particles and, by so doing, re-

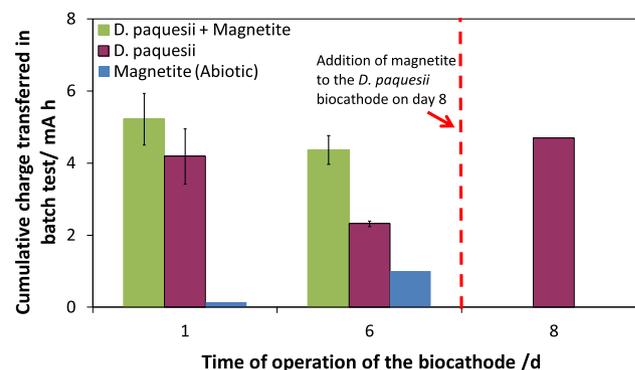


Fig. 3 – Cumulative charge transferred during chronoamperometry (CA) batch tests carried out on the different (bio)cathodes on day 1, 6 and 8. Error bars represent the standard deviation of replicated experiments.

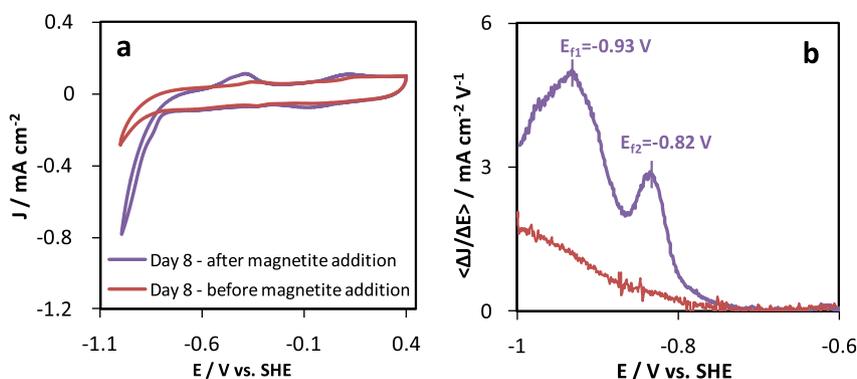


Fig. 4 – Effect of magnetite addition to the 8-day old *D. paquesii* biocathode. (a) Cyclic voltammetry (scan rate: 10 mV/s). (b) First derivative of the cathodic current.

establishing the electron transfer capabilities of the *D. paquesii* biofilm.

In addition to CA tests, CV was also used to characterize the effect of magnetite addition to the 8-day old (non-amended) biocathode that had lost most of its electrocatalytic activity. The CV profile and its first derivative recorded just before the addition of magnetite nanoparticles, revealed substantially reduced cathodic currents (-0.25 mA/cm^2 at -1000 mV) and the lack of the two peaks (Fig. 4), which seemed to characterize the bioelectrocatalytic activity of *D. paquesii*.

Remarkably, the addition of magnetite nanoparticles not only resulted in increased cathodic currents (-0.75 mA/cm^2 at -1000 mV) but also in the reappearance of the two characteristic redox peaks (Fig. 4(b)), even though at slightly more reducing formal potentials (E_{f1} and E_{f2} at -930 and -820 mV , respectively). Possibly, this shift was somehow due to the above-mentioned passivation phenomena, which introduced additional resistances to electron transfer processes.

Conclusion

In conclusion, this study demonstrated that addition of small amounts (ca. 10 mg/L or 0.16 mg/cm^2 of cathode surface) of electrically-conductive magnetite nanoparticles is a viable and effective strategy to enhance the electrocatalytic activity and improve the resilience of a model (H_2 -producing) microbial biocathode. Further studies would include longer-term experiments (weeks) in order to assess stability through time and if there's any negative effect of magnetite addition.

Although the mechanism underlying the stimulatory effect of magnetite nanoparticles remains, at least partially, unknown it is likely that magnetite particles allow increasing the electrical conductivity of the biofilm–electrode interface, thereby enhancing the EET processes. Taking into consideration that previous studies have already shown that magnetite nanoparticles allow accelerating EET processes in mixed microbial cultures bringing about a variety of metabolic reactions, it is plausible that the mode of action of the particles is rather unspecific and could be extended to other bioelectrocatalytic reactions.

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